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Age-Related Effects of Varying Ammonia Concentrations on Hematophysiological Variables in Broiler Chickens

H.A. Olanrewaju*, J.L. Purswell, S.D. Collier and S.L. Branton
Poultry Research Unit, Agricultural Research Service, USDA, P.O. Box 5367,
Mississippi State, MS 39762-5367, USA

Abstract: This study examined the response of different aged birds of the same genetic strain exposed to ammonia (NH_3) at set concentrations on blood gases, electrolytes and acid-base balance under environmentally controlled conditions. The experiment consisted of a 4×4 factorial with a randomized design. The 16 treatments consisted of 4 levels (0, 25, 50 and 75 ppm) of NH_3 concentrations and 4 different ages (1-d, 7-d, 14-d and 21-d) of birds. Venous blood samples were collected at the end of each 7 d of atmospheric NH_3 exposure. Partial pressure of CO_2 (pCO_2), pH, Hematocrit (Hct) and Hemoglobin (Hb) increased significantly ($p \leq 0.05$), whereas partial pressure of O_2 (pO_2), bicarbonate (HCO_3^-) and K^+ decreased with increasing NH_3 concentration compared with 0 ppm. In addition, pO_2, pCO_2, HCO_3^-, Hct, Hb, Na^+ and Anion gap (Angap) increased significantly ($p \leq 0.05$), while pH, glucose and corticosterone decreased as bird's age increased. Ammonia x age interactions were observed for pH, anion gap and HCO_3^-. Plasma corticosterone concentrations were significantly different for age and were not affected by NH_3. The effect of age was more pronounced than that of NH_3 on examined variables. This effect of age on examined blood physiological variables improved as the age of birds increased from 1-d to 21-d old birds. Most blood physiological variables of different aged birds of the same genetic strain respond differently to set NH_3 concentrations of 0 to 75 ppm and younger birds have a more intense reaction to the NH_3 than older birds.

Key words: Ammonia, age, acid-base balance, broiler, well-being

INTRODUCTION

The trend toward confinement housing for broiler chickens has increased the problems associated with a variety of atmospheric contaminants including ammonia (NH_3), carbon dioxide and dust and as a consequence, NH_3 has been studied more than other air pollutants (Miles *et al*., 2004, 2006; Olanrewaju *et al*., 2007, 2008a). Atmospheric NH_3 has been shown to be detrimental to poultry health and performance (Carlile, 1984; Kristensen and Wathes, 2000) and is cited as an environmental concern as well (NRC, 2003; USDA, 2005). Exposure to high concentrations of NH_3 (>50 ppm) causes keratoconjunctivitis, with symptoms including watery eyes, closed eyelids, rubbing of eyes with the wings and blindness (Bullis *et al*., 1950; Faddoul and Ringrose, 1950). Our recent research evaluated the interactive effects of inhalation of ambient air with elevated NH_3 concentrations and different light intensities on blood physiological variables and welfare in broiler chickens under environmentally controlled conditions. The results indicated that exposure of broiler chickens to aerial NH_3 concentrations of 0-50 ppm from d 1-14 posthatch in the presence of light intensities ranging from 0.2-20 lx had no direct effect on some physiological blood variables and did not induce stress in broilers. However, the interaction of bird age and level of NH_3 exposure has not been addressed. Therefore, the objective of the present study was to examine the age-related effects of atmospheric NH_3 exposure on blood gases, electrolytes, acid-base balance and welfare of a single strain of broiler chickens under environmentally controlled conditions. We hypothesized that different aged birds of the same genetic strain will respond differently to set NH_3 concentrations and younger birds will have a more intense reaction to the NH_3 than older birds.

MATERIALS AND METHODS

Bird husbandry: A total of 768 (384 males/384 females) 1-d-old Ross × Ross 708 chicks purchased from a commercial hatchery (Aviagen Inc., Huntsville, AL) were used for this study. Chicks were vaccinated for Marek's, Newcastle and Infectious bronchitis diseases at the hatchery. Birds were reared in small gas exposure boxes (Fig. 1) measuring 1.0 m W × 1.0 m D × 0.76 m H located inside large environmentally controlled chambers. Each box contained fresh pine shavings and was equipped with a tube feeder and nipple drinker line. Positive-pressure ventilation was provided for each box with a 120 cm fan and exhausted outside the environmental chamber. Light, temperature and humidity set points were maintained as scheduled through out the study period. Ammonia gas injection was regulated with a rotameter installed on each box. Diets were formulated to meet or exceed NRC (1994) nutrient recommendations. Feed and water were offered *ad libitum*. Mortalities were weighed and recorded daily.

Fig. 1: Gas exposure box measuring 1.0 m W × 1.0 m D × 0.76 m H. One box for each level (0, 25, 50 and 75 ppm) of ammonia concentration was placed inside four large environmental chambers described by Reece and Deaton (1969)

Treatments: Sixteen treatments (4x4 factorial) were evaluated. The treatments consisted of inhalation of 0, 25, 50 or 75 ppm of NH_3 for 7 d on 4 different ages (1-d, 7-d, 14-d and 21-d old) of equal male and female broiler chickens. Procedures for NH_3 administration were similar to those used in previous NH_3 studies (Olanrewaju *et al*., 2007, 2008a). Anhydrous NH_3 (25, 50 and 75 ppm) was continuously metered into 3 of the 4 boxes in each chamber through panel-mount flow meters. No NH_3 (0 ppm) was added to the remaining 1 box in each chamber that served as controls. To eliminate NH_3 contribution from used litter, birds were placed on fresh pine shavings that were 10 cm deep at the beginning of each week of ammonia application.

Measurements: Ammonia was measured daily at 0800, 1200, 1600 and 2000 h daily and after disturbing the chamber atmosphere by animal caretakers with an electrochemical gas monitor (Pac III, Drager safety, Inc., Pittsburgh, PA). During the 1 week ammonia exposure period on the 4 different ages, the average measured NH_3 concentration for each treatment level approximated the designated level, but variability with concentration increased due to the association of atmospheric NH_3 with that excreted in the shavings. The NH_3 levels ranged from 0-1.4 ppm for the control treatment (0 ppm), 25.3-28.5 ppm for the 25-ppm treatment, 48.8-56.6 ppm for the 50-ppm and 70.0-82.6 for 75-ppm treatment. The average actual concentrations for the 25-, 50- and 75-ppm treatments were 26.82, 53.22 and 76.77 ppm, respectively.

Blood collection and chemical analyses: At the end of each week, 6 chicks (3 males/3 females) from each box per chamber for a total of 24 birds per treatment/age were randomly selected for assessment of their general welfare and blood sample collection. Blood samples were collected between 0800 and 0900 h on sampling d from a brachial vein of 6 randomly selected birds from each box and the birds were then returned to the appropriate box by using our standard handling procedure (Olanrewaju *et al*., 2007, 2008a). In addition, unnecessary discomfort to the birds was avoided by using proper housing and handling techniques, as described by the NRC (1996). Blood samples (4 cc) were collected directly into heparinized (50 IU/mL) monovette syringes. All bleedings were completed within 45 s after birds were caught. Blood samples were drawn directly from the syringes into a blood gas electrolyte analyzer (ABL-80 Flex, Radiometer America, Westlake, OH) for immediate analysis of partial pressure of CO_2 (pCO_2), partial pressure of O_2 (pO_2), pH, Hematocrit (Hct), Hemoglobin (Hb), Anion gap (Angap) and electrolytes (Na^+, K^+, Ca^{2+}, HCO_3^- and Cl^-). The pH, pCO_2, pO_2 and HCO_3^- values were corrected to reflect a body temperature of 41.5°C (Burnett and Noonan, 1974). The needle mounted on each monovette syringe was then removed, a cap was placed over the needle port and the syringes containing the blood samples were submerged into ice.

The remaining iced blood samples were transferred to the laboratory, centrifuged at 4,000 × *g* for 20 min and the packed blood cells were expelled from the syringes. The plunger on each monovette was broken off and the syringe served as a storage vial for the remaining plasma. This procedure ensured that the plasma samples were never exposed to ambient air. Plasma samples were stored at -20°C for later chemical analyses. Plasma samples were removed from the freezer, thawed and each sample was analyzed for Corticosterone (CS) and Glucose (GLU) as described in previous NH_3 study (Olanrewaju *et al*., 2008a).

Statistical analyses: A 4×4 factorial with a randomized design was used in this study. Box was considered the experimental unit with chamber being the replicate. The 16 treatments consisted of 4 levels of NH_3 concentrations × 4 different ages. The main effects of NH_3, age and the interaction of these 2 factors on physiological variables were tested by using the MIXED procedure of SAS (SAS Institute, 2004). Means comparisons were assessed by least significant differences and statements of significance were based on $p \leq 0.05$.

RESULTS

The influence of atmospheric NH_3 exposure, age and their interaction on blood gases, hematocrit and hemoglobin are in Table 1. There were significant ($p \leq 0.05$) main effects of NH_3 and age on blood gases,

Table 1: Age-related effects of ammonia on blood chemistry in broiler chickens

Item	pH	pCO_2 (mmHg)	O_2 (mmHg)	HCO_3^- (mmHg)	Hct (%)	Hb (g/dL)
Ammonia treatment						
0 ppm	7.31^{b}	54.5^{b}	65.0^{a}	28.7^{a}	23.4^{b}	8.2^{b}
25 ppm	7.31^{b}	55.9^{b}	59.6^{b}	27.5^{b}	24.3^{b}	8.3^{b}
50 ppm	7.35^{a}	58.4^{a}	61.3^{b}	27.7^{b}	25.9^{a}	8.3^{ab}
75 ppm	7.34^{a}	57.2^{a}	60.3^{b}	27.1^{b}	26.3^{a}	8.4^{a}
Age						
1-d old	7.35^{a}	48.5^{c}	52.9^{c}	26.3^{b}	24.6^{c}	7.9^{c}
7-d old	7.32^{b}	55.3^{b}	59.9^{b}	27.6^{a}	25.4^{bc}	8.2^{bc}
14-d old	7.28^{c}	59.2^{a}	66.3^{a}	27.0^{ab}	27.1^{a}	8.7^{a}
21-d old	7.30^{bc}	58.8^{a}	65.5^{a}	28.1^{a}	26.7^{ab}	8.6^{ab}
SEM[1]	0.007	0.828	1.230	0.326	0.355	0.118
Ammonia-intensity treatment						
0 ppm-1d old	7.34^{abcd}	47.9	60.9	25.9^{ab}	23.3	7.5
0 ppm-7 d old	7.32^{abcd}	54.8	62.2	27.4^{ab}	25.2	8.1
0 ppm-14 d old	7.30^{cd}	56.7	68.5	27.9^{ab}	24.8	8.4
0 ppm-21 d old	7.29^{cd}	58.7	65.3	27.5^{ab}	24.4	8.3
25 ppm-1 d old	7.32^{bcd}	51.5	53.3	25.6^{bc}	25.6	8.2
25 ppm-7 d old	7.35^{abc}	54.0	57.6	29.2^{a}	25.8	8.3
25 ppm-14 d old	7.29^{cd}	58.8	67.0	27.0^{ab}	24.7	8.6
25 ppm-21 d old	7.30^{bcd}	59.2	67.6	28.2^{ab}	24.1	8.7
50 ppm-1 d old	7.49^{a}	57.3	53.9	28.0^{ab}	24.6	7.9
50 ppm-7 d old	7.31^{bcd}	56.8	57.6	27.3^{ab}	25.2	8.1
50 ppm-14 d old	7.27^{d}	61.4	67.0	27.1^{ab}	27.1	8.7
50 ppm-21 d old	7.30^{cd}	59.7	67.6	28.3^{ab}	26.6	8.6
75 ppm-1 d old	7.47^{ab}	58.2	53.9	26.3^{ab}	25.0	8.0
75 ppm-7 d old	7.30^{cd}	55.8	57.9	26.4^{ab}	25.6	8.2
75 ppm-14 d old	7.28^{d}	59.9	65.6	26.9^{ab}	27.8	8.8
75 ppm-21 d old	7.41^{bcd}	57.7	63.8	28.3^{ab}	26.8	8.5
SEM[2]	0.015	1.656	2.460	0.651	0.710	0.237
Source of variation	----------P-value----------					
Ammonia	0.0495	0.0448	0.0479	0.0187	0.0466	0.0517
Age	0.0001	0.0001	0.0001	0.0026	0.0001	0.0001
Ammonia × Age	0.0053	0.4947	0.3421	0.0507	0.9094	0.9084

[ab]Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$), [1]Pooled SEM for main effects (n = 16)
[2]Pooled SEM for interaction effect (n = 4).

hematocrit and hemoglobin, especially for the 50-ppm and 75-ppm levels of NH_3 exposure. The Main Effect Mean (MEM) for NH_3 was significant for pH, pCO_2, Hct, Hb, which were increased and for pO_2 and HCO_3^- that were decreased all with increase in NH_3 concentration (Table 1). The MEM for age was significant for pCO_2, pO_2, HCO_3^-, Hct, Hb and for pH especially that of 1-d-old compared with that of 21-d-old groups. There was significant NH_3 by age interactions for pH and HCO_3^- (Table 1).

The MEM for blood levels of K^+ was only significantly lowered by NH_3 (Table 2). In addition, the MEM for Ca^{2+}, K^+, Na^+, Cl^- and angap were significantly different by age. There was significant NH_3 by age interactions for angap. Age significantly affected MEM for plasma concentrations of Glucose (GLU) and Corticosterone (CS) as shown in Table 3. Plasma concentrations of GLU and CS of 1-d-old birds were significantly higher compared with that of 21-d-old birds. There was no significant main effect of NH_3 on GLU, CS, CHOL, TRIG and McHc. Furthermore, there was no significant effect of age on CHOL, TRIG and McHc. There was no interactive effect of NH_3 x age on any of the variables (Table 3).

DISCUSSION

Our findings clearly show that different aged birds of the same genetic strain respond differently to set NH_3 concentrations of 0-75 ppm on most examined blood physiological variables and younger birds have a more intense reaction to the ammonia than older birds. Results suggest an increased respiratory rate in younger broilers exposed to greater levels of NH_3-. This may be attributed to mild metabolic acidosis, which has been associated with a minor reduction of pH and of HCO_3- concentration, along with sustained plasma Ca^{2+} as we reported earlier (Olanrewaju *et al*., 2008a). The pH of the blood is maintained within a very narrow range because sudden changes can result in cellular damage via protein ionization (Eckert, 1988). The distribution of NH_3 between body compartments is strongly influenced by pH and transfer of NH_3 is dependent upon arterial blood pH and systemic alkalosis exacerbates NH_3 toxicity (Ott and Larsen, 2004). The finding that the pH was elevated and plasma pCO_2 was changed indicates minimal metabolic alkalosis. The increased pCO_2 observed in this study, which is similar to our previous

Table 2: Age-related effects of ammonia on blood chemistry in broiler chickens

Item	Ca^{2+} (mEq/L)	K^+ (mEq/L)	Na^+ (mEq/L)	Cl^- (mEq/L)	Angap (mmol/L)
Ammonia treatment					
0 ppm	3.20	5.39^{a}	148	108	19.6
25 ppm	3.22	5.12^{a}	147	107	19.5
50 ppm	3.22	5.09^{b}	149	109	18.8
75 ppm	3.17	4.77^{b}	147	106	19.0
Age					
1-d old	3.20^{ab}	5.15^{a}	144^{b}	107^{ab}	17.8^{c}
7-d old	3.13^{b}	4.72^{b}	145^{b}	104^{b}	18.9^{bc}
14-d old	3.25^{a}	5.12^{a}	150^{a}	110^{a}	20.2^{a}
21-d old	3.23^{ab}	4.97^{ab}	151^{a}	109^{a}	20.0^{ab}
SEM[1]	0.0276	0.092	0.680	1.116	0.306
Ammonia-intensity treatment					
0 ppm-1d old	3.25	5.22	145	107	20.4^{a}
0 ppm-7 d old	3.12	5.19	146	107	18.4^{ab}
0 ppm-14 d old	3.20	5.34	149	110	19.6^{a}
0 ppm-21 d old	3.23	5.20	150	109	20.0^{a}
25 ppm-1 d old	3.23	5.49	144	106	19.8^{a}
25 ppm-7 d old	3.14	4.53	145	105	17.5^{abc}
25 ppm-14 d old	3.27	5.26	150	109	20.4^{a}
25 ppm-21 d old	3.23	4.88	150	109	20.2^{a}
50 ppm-1 d old	3.21	5.09	144	108	15.2^{c}
50 ppm-7 d old	3.15	4.92	145	105	20.0^{a}
50 ppm-14 d old	3.30	5.30	152	112	20.5^{a}
50 ppm-21 d old	3.23	5.05	152	111	20.0^{a}
75 ppm-1 d old	3.14	4.81	144	107	16.1^{bc}
75 ppm-7 d old	3.13	4.55	146	98	20.1^{a}
75 ppm-14 d old	3.23	4.97	149	109	20.2^{a}
75 ppm-21 d old	3.21	4.74	149	108	19.9^{a}
SEM[2]	0.053	0.183	1.360	2.232	0.612
Source of variation	----------P-value----------				
Ammonia	0.6150	0.0536	0.4444	0.2190	0.2371
Age	0.0228	0.0071	0.0001	0.0018	0.0001
Ammonia × Age	0.9530	0.5338	0.8861	0.8046	0.0001

^{ab}Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$), [1]Pooled SEM for main effects (n = 16)
[2]Pooled SEM for interaction effect (n = 4).

report (Olanrewaju *et al*., 2008b) may account for respiratory hyperpnea, which fails to prevent pO_2 from decreasing. However, decreased pO_2 may account for the metabolic acidosis because of anaerobic glycolysis or accumulations of carbonic and other intracellular acids. Acid-base disturbances may be a consequence of polypnea or panting, leading to hyperventilation and elimination of CO_2, which may result in hypocapnic alkalosis. Increases in Hb and Hct, along with reduced blood oxygen saturation, as observed in this study, may be related to the increased metabolic activity needed to meet the energy demands for growth and especially for maintenance under NH_3 inhalation stressful conditions, leading to an increase in erythropoiesis as a compensatory reaction to the lack of oxygen in the tissues. However, based on our previous studies (Olanrewaju *et al*., 2008a, 2008b) this current findings data further confirmed that the pulmonary vascular capacity of modern broilers is marginally adequate to accommodate the Cardiac Output (CO) required to support and sustain the respiratory and metabolic demand for rapid growth. Beker *et al*. (2004) reported in broilers that Hct increased with age, but was not affected by atmospheric NH_3 concentration. It has been reported that increased Hct occurred in response to hypoxia, which suggested a physiologic attempt to overcome the hypoxic condition by increasing the number of red blood cells (Maxwell *et al*., 1990).

The essential electrolytes for the maintenance of the acid-base balance are Sodium (Na^+), Potassium (K^+) and Chlorine (Cl^-). However, K^+ is more involved in many metabolic processes, including the acid-base balance (Borges *et al*., 2007). It has been shown that absorbed NH_3 is well distributed throughout body compartments and reacts with hydrogen ions to produce ammonium ions (IPCS, 1990). In agreement with our findings, plasma K^+ concentration has been reported to decrease because ammonium ions compete with potassium ions for inward transport over the cytoplasmic membrane, via K^+ transport proteins like the Na^+/K^+ -ATPase and the $Na^+K^+2Cl^-$ cotransporter because of similar ionic radius of hydrated ammonium and K^+ (Martinelle and Haggstrom, 1993).

Table 3: Age-related effects of ammonia on blood chemistry in broiler chickens

Item	GLU (mg/dL)	CS (pg/mL)	CHOL (mg/dL)	TRIG (mg/dL)	McHc (%)
Ammonia treatment					
0 ppm	225	2109	127	89.8	32.2
25 ppm	227	1967	134	95.9	32.2
50 ppm	231	1751	133	95.0	32.2
75 ppm	231	1759	135	86.8	32.2
Age					
1-d old	231[a]	3413[a]	132	93.6	32.1
7-d old	234[a]	1504[b]	134	97.3	32.2
14-d old	231[a]	1749[b]	133	81.8	32.2
21-d old	219[b]	1419[b]	131	94.9	32.2
SEM[1]	2.012	413.55	2.22	4.234	0.022
Ammonia-intensity treatment					
0 ppm-1day old	222[abc]	2650	125	98.1	32.1
0 ppm-7 d old	236[ab]	1495	133	88.8	32.2
0 ppm-14 d old	229[abc]	3529	124	80.3	32.2
0 ppm-21 d old	213[c]	2764	128	92.3	32.2
25 ppm-1 d old	225[abc]	4224	139	97.4	32.2
25 ppm-7 d old	229[abc]	1261	133	94.9	32.2
25 ppm-14 d old	232[abc]	1359	137	94.5	32.2
25 ppm-21 d old	220[bc]	1025	127	96.9	32.2
50 ppm-1 d old	242[a]	1847	124	89.7	32.1
50 ppm-7 d old	236[ab]	1546	138	110.0	32.1
50 ppm-14 d old	232[abc]	1845	133	79.4	32.2
50 ppm-21 d old	224[abc]	1764	138	101.0	32.1
75 ppm-1 d old	233[ab]	2932	139	89.2	32.1
75 ppm-7 d old	237[ab]	1714	131	95.8	32.1
75 ppm-14 d old	231[abc]	1265	139	72.9	32.2
75 ppm-21 d old	220[bc]	1125	131	89.4	32.2
SEM[2]	4.025	827.100	4.442	8.467	0.045
Source of variation	----------P-value----------				
Ammonia	0.0572	0.0552	0.0772	0.3853	0.5391
Age	0.0001	0.0037	0.7770	0.0579	0.0746
Ammonia × Age	0.2482	0.6889	0.0950	0.7603	0.6721

[ab]Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$), [1]Pooled SEM for main effects (n = 16)
[2]Pooled SEM for interaction effect (n = 4).

Significant increases in blood glucose and CS decreased as age of the birds increased further demonstrating the detrimental effect of NH_3 on younger birds. Investigators have reported alterations of some intermediate metabolism during NH_3 intoxication, suggesting that hormone action is also changed (Chalupa and Opliger, 1969; Garwacki *et al*., 1978). It has been shown that certain metabolites of intermediary metabolism, such as glucose and Nonesterified Free Fatty Acids (NFFA) increased when blood NH_3 is elevated (Chalupa and Opliger, 1969). Other evidence suggests that the metabolic response to NH_3 may be partially due to epinephrine (Garwacki *et al*., 1978). A strong positive correlation between age and inhaled NH_3 in humans indicated a decrease in upper airway reflex sensitivity with increasing age (Erskine *et al*., 1993). The same study reported that younger individuals exhibited a higher upper airway reflex sensitivity to NH_3 than that of older test subjects (Erskine *et al*., 1993). In this study, blood CS decreased linearly as age increased for 1d-14d and it decreased significantly on 21-d old birds. Physiologically, all offspring including younger birds are highly vulnerable to chemical toxicants and more susceptible to NH_3 inhalation because their immune system is not fully functional and due to suppressive effect of NH_3 on immune system. Young offspring can not counteract toxic effects as well as an adult can. Their metabolic pathways, especially in the first week of life, are immature. Their ability to metabolize, detoxify and excrete many chemicals differs from adults. Therefore, exposure of younger birds to high levels of NH_3 such as 75 ppm in air may be more detrimental to them. Animal studies have revealed that NH_3 affects the immune system (Mulloy and Visek, 1980). Some studies indicate that NH_3 can increase susceptibility to pathogens (Anderson *et al*., 1964; Broderson *et al*., 1976; Schoeb *et al*., 1982; Targowski *et al*., 1984). For instance, the severity of mycoplasmal, viral and secondary bacterial infections of the respiratory tract has been enhanced in simple-stomached species exposed to concentrations of NH_3 that occur in usual environments (Anderson *et al*., 1964; Broderson *et al*., 1976; Visek, 1981). Furthermore,

a significant increase in the severity of respiratory signs characteristic of murine respiratory mycoplasmosis was observed in rats exposed to NH_3 at 25 ppm for 4-6 weeks followed by inoculation with *Mycoplasma pulmonis* intranasally (Broderson *et al*., 1976). We conclude that different aged birds of the same genetic strain respond differently to set NH_3 concentrations of 0-75 ppm on most examined blood physiological variables and younger birds have a more intense reaction to the NH_3 than older birds.

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